Marking embryonic stem cells with a 2A self-cleaving peptide: a NKX2-5 emerald GFP BAC reporter.

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Public Summary:

Scientific Abstract:
BACKGROUND: Fluorescent reporters are useful for assaying gene expression in living cells and for identifying and isolating pure cell populations from heterogeneous cultures, including embryonic stem (ES) cells. Multiple fluorophores and genetic selection markers exist; however, a system for creating reporter constructs that preserve the regulatory sequences near a gene's native ATG start site has not been widely available. METHODOLOGY: Here, we describe a series of modular marker plasmids containing independent reporter, bacterial selection, and eukaryotic selection components, compatible with both Gateway recombination and lambda prophage bacterial artificial chromosome (BAC) recombineering techniques. A 2A self-cleaving peptide links the reporter to the native open reading frame. We use an emerald GFP marker cassette to create a human BAC reporter and ES cell reporter line for the early cardiac marker NKX2-5. NKX2-5 expression was detected in differentiating mouse ES cells and ES cell-derived mice. CONCLUSIONS: Our results describe a NKX2-5 ES cell reporter line for studying early events in cardiomyocyte formation. The results also demonstrate that our modular marker plasmids could be used for generating reporters from unmodified BACs, potentially as part of an ES cell reporter library.